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Spermatid polarity is regulated by the actin- and microtubule (MT)-based cytoskeletons

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Abstract

It is conceivable that spermatid apico-basal polarity and spermatid planar cell polarity (PCP) are utmost important to support spermatogenesis. The orderly arrangement of developing germ cells in particular spermatids during spermiogenesis are essential to obtain structural and nutrient supports from the fixed number of Sertoli cells across the limited space of seminiferous epithelium in the tubules following Sertoli cell differentiation by ~17 day postpartum (dpp) in rodents and ~12 years of age after puberty in humans. Yet few studies are found in the literature to investigate the role of these proteins to support spermatogenesis. Herein, we briefly summarize recent findings in the field, in particular emerging evidence that supports the concept that apico-basal polarity and PCP are conferred by the corresponding polarity proteins through their effects on the actin- and microtubule (MT)-based cytoskeletons. While much research is needed to bridge our gaps of understanding cell polarity, cytoskeletal function, and signaling proteins, a critical evaluation of some latest findings in the field as summarized herein provides some important and also thought-provoking concepts to design better functional experiments to address this important, yet largely expored, research topic.

Introduction

During spermatogenesis in the mammalian testis, including both rodents and humans, developing spermatids display unusual polarity to support the packaging of millions of spermatids across the seminiferous epithelium. Thus, millions of spermatozoa can be

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produced daily in the limited space of the seminiferous tubules tightly packed inside the testes [1–3]. Studies have shown that there are two types of spermatid polarity during the epithelial cycle to support spermatogenesis. The first type is the apico-basal polarity in which the heads of elongating/elongated spermatids in the testis are orientated by pointing to the basement membrane (i.e., individual cell polarity), which is supported by the partitioning defective (Par)-[4], the Scribble- [5], and the Crumbs (Crb)- [6] based polarity protein complexes that are found in virtually all mammalian cells besides Sertoli and/or germ cells in the testis [7, 8]. The second type is the spermatid planar cell polarity (PCP) in which polarized elongating/elongated spermatids are aligned across the plane of the Sertoli cell epithelium in the tubules by orientated unidirectionally, supported by PCP proteins such as Vangl2 [9, 10]. Studies have shown that these polarity proteins and PCP proteins are working in concert with F-actin-based cytoskeleton to support spermatid polarity and PCP [2, 11]. However, emerging evidence based on published findings in the testis has shown that the microtubule (MT)-based cytoskeleton is also involved in polarity protein- and PCP protein-mediated spermatid polarity. A recent review in this Special Issue [11] has summarized recent findings regarding the role of the Par-, the Scribble- and the Crumbs homolog 3 (Crb3)-based polarity protein complexes in the adult rat testis by working closely with the F-actin-based cytoskeleton to modulate spermatid polarity. As such, we do not include such discussion and pertinent findings herein to avoid redundancy. Instead, we focus more on latest findings using different animal models to assess the relationship between spermatid polarity/spermatid PCP and changes in the organization of actin- and MT-based cytoskeletons. This information should provide insightful information regarding future experimental planning to better understand the integrated function of both cytoskeletons to support spermatid polarity and PCP.

Polarity proteins and planar cell polarity proteins

Studies in the rat testis have shown that, similar to other epithelial cells, the Par-based polarity complex is comprised of at least 4 proteins: Par3, Par6, aPKC, and Cdc42 which tightly associate with the integral membrane protein JAM-C (junctional adhesion molecule C, also known as JAM-1) (Table 1), predominantly expressed at the apical ES (ectoplasmic specialization) to modulate apico-basal spermatid polarity and adhesion [4] at the Sertoli cell-spermatid interface [4]. However, the Par-based proteins are also expressed at the basal ES at the Sertoli cell-cell interface near the basement membrane, consistent with its localization at the blood-testis barrier (BTB) [4]. In this context, it is of interest to note that the ES is a testis-specific adherens junction (AJ) type, restrictively expressed at the Sertoli-spermatid interface, limited to step 8–19 spermatids in the rat testis, whereas the basal ES is only found at the Sertoli cell-cell interface, coexisting with the tight junction (TJ) to create the Sertoli cell BTB [12–14]. The Par-based polarity complex is working closely with the Crb3-based polarity complex which is composed of Crb3 (an integral membrane protein), Pals1 and PatJ [6] to support apico-basal polarity as noted in other epithelia [15]. For instance, studies have shown that aPKC in the Par-based polarity protein can modulate Par3 or Crb3 function via phosphorylation, inducing the necessary cross-talk between these two polarity complex to modulate cell polarization [15–17]. On the other hand, the Scribble-based polarity complex that supports apico-basal polarity is composed of Scribble, Lgl2

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(Lethal giant larvae 2) and Dlg1 (Discs large 1) in the rat testis [5], which is mutually exclusive regarding its function and also physical localization *vs.* the Par- and the Crb3-based polarity complexes [7, 15]. In the testis, Scribble is expressed predominantly at the basal ES in virtually all stages of the epithelial cycle, however, its expression at the apical ES is limited to stage VII–VIII tubules [5]. Interestingly, Crb3 is only expressed at the basal ES/BTB in stage I–VIII tubules [6]. As noted in Table 1, the function of each of these polarity protein complexes and their partner proteins has been evaluated based on studies of genetic models. Interestingly, their functional significance in the testis to support spermatid polarity and/or spermatogenesis remains largely unknown. Studies performed in the testis have noted that these polarity proteins that confer Sertoli and germ cell apico-basal polarity exert their effects through the actin-based cytoskeleton [4–6]. It is expected that MTs are involved in spermatid apico-basal polarity since the apical ES function is mutually supported by both the actin- and MST-based cytoskeletons [18–20] and both actin filaments and MT protofilaments are adjacent to each other at the apical ES [12, 21]. Nonetheless, the involvement of MT in spermatid apico-basal polarity in the testis remains to be investigated.

The NC1 (non-collagenous domain 1 of collagen α3 (IV) chain) domain model

In the testis, the Sertoli and germ cells that constitute the seminiferous epithelium is considered to be an immune privilege site in particular the adluminal compartment behind the BTB [22–24]. This is due to the Sertoli cell-cell junctions, in particular the TJ and the basal ES, near the base of the epithelium, adjacent to the basement membrane, that create the BTB which deny the entry of other cells, such as macrophages and fibroblasts, and other biological and physiological substances including electrolytes, mineral salts and biomolecules into the adluminal (apical) compartment (Figure 1). However, recent studies have shown that one of the major building blocks of the basement membrane, the collagen α3 (IV) chain, is a regulator of the basal ES (i.e., BTB) and also the apical ES function. For instance, the inclusion of an anti-collagen type IV chain antibody obtained commercially in primary Sertoli cell cultures in vitro with an established TJ-barrier was found to perturb the Sertoli cell TJ-permeability barrier function [25]. This observation thus supports the notion that the collagen α3 (IV) chain in the basement membrane is playing a role in modulating the Sertoli cell barrier function at the BTB. This finding is also consistent with an earlier report that the use of antibodies prepared against the seminiferous tubule basement membrane induced extensive seminiferous epithelial damage including germ cell exfoliation, grossly disrupting spermatogenesis [26]. Subsequent studies have shown that such damages are mediated by the non-collagenous fraction of the basement membrane [27]. Additionally, studies from other epithelia have shown that the non-collagenous domains of collagen chains, in particular NC1 domain (non-collagenous domain 1) residing at the N-terminus when cleaved from collagen chains, act as biologically active peptides to modulate cell adhesion function and other biological activities (e.g., angiogenesis) in mammalian cells and tissues [28, 29]. We thus cloned the NC1 domain and obtained the recombinant protein against the NC1 domain at the N-terminal region of collagen α (IV) chain, a peptide of ~30 kDa, and noted that the purified recombinant protein was a potent biologically active peptide to induce Sertoli cell BTB restructuring [30]. Overexpression of the NC1 domain peptide

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using a mammalian expression vector pCI-neo using the Polyplus in vivo-jetPEI as a transfection medium (with a transfection efficiency at ~50–60%) in the testis in vivo was found to induce Sertoli BTB function disruption using a biotin-based BTB integrity assay in vivo [31], consistent with the findings in vitro that overexpression of NC1 domain in Sertoli cells [31] or inclusion of the recombinant NC1 domain peptide in Sertoli cell cultures [30] perturbed the Sertoli cell TJ-permeability barrier function. Additionally, it was of interest to note that overexpression of the NC1 domain in the testis in vivo also perturbed the apical ES function via a gross disruption on the organization of both actin- and MT-based cytoskeletons at the site [31]. Since the spatial expression and distribution of apical ES proteins β1-integrin [32–34] and laminin-γ3 chain [34, 35] were grossly disrupted [31], illustrating the apical ES function was perturbed. In fact, overexpression the NC1 domain in the testis in vivo led to extensive germ cell exfoliation, in particular elongating/elongated spermatids, such as within 7 days after NC1 domain overexpression when many tubules had contained only Sertoli cells and primitive germ cell types (e.g., spermatogonia, early spermatocytes) [31] since the loss of apical ES failed to support elongating and elongated spermatid adhesion onto the epithelium. In fact, over 50% of the tubules were devoid of elongating/elongated spermatids; the reason that not all tubules were affected is likely due to the transfection efficiency which was to be ~50–60% instead of >95% [31]. More important, numerous spermatids remained trapped deep inside the seminiferous epithelium and many of these elongated spermatids had defects in their polarity wherein their heads no longer aligned by pointing toward the basement membrane, but deviated by 90° to 180° from the intended orientation noted in control testes [31]. In short, even in the absence of functional apical ES, spermatids failed to be transported to the epithelium near the tubule lumen to undergo spermiation, and the non-funcitonal apical ES failed to support spermatid polarity. It is of important to note that the disruptive effects of NC1 domain on the basal ES/BTB and the apical ES function was reversible, since spermatogenesis gradually resumed and virtually all of the affected tubules repopulated with all germ cell types by day 45 [31].

A detailed analysis on the status of spermatogenesis in the NC1 domain peptide affected tubules following its overexpression have noted that many of the step 19 spermatids remained trapped deep inside the seminiferous epithelium long after spermiation, even found in stage IX–XII tubules, and most of these spermatids had defects in polarity [31]. For instance, the heads of these elongated spermatids no longer pointed toward the basement membrane as noted in control and normal testes, but deviated by 90° to 180° from the intended orientation [31]. Additionally, the organization of F-actin and MT surrounding these elongated spermatids with defects in polarity were grossly disrupted, either missing altogether or diffusely localized, thereby failing to support spermatid polarity and adhesion [31]. Specifically, the track-like structures conferred by MTs that laid perpendicular to the basement membrane as noted in control testes were virtually non-detected in all the affected tubules that had defects in spermatogenesis following NC1 domain overexpression [31]. For instance, the MT-conferred tracks were extensively truncated and virtually no tracks were detected that ran from the basement membrane to the adluminal compartment near the tubule lumen across the entire epithelium in tubules following overexpression of NC1 domain as noted in control testes [31]. Collectively, these data thus support the notion that

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spermatid polarity (and also adhesion) are tightly associated with the integrity of actin- and MT-based cytoskeletons.

In this context, it is of interest to note that while defects of spermatid polarity noted in this NC1 model is likely contributed by the three polarity protein complexes involving the actin- and MT-based cytoskeletons, some recent observations suggest that the third cytoskeleton, namely the vimentin-based intermediate filament, may also be involved. For instance, as noted in Figure 2, the polarity protein Crb3 was only partially co-localized with F-actin in the seminiferous epithelium, however, Crb3 almost localized superimposable with vimentin (Figure 3). While much work is needed to define the role and involvement of intermediate filament-based cytoskeleton on spermatid polarity, the observations noted in Figures 2 and 3 have shown that such an expanded is much needed in future investigation.

The Vangl2 (Van Gogh-like 2) model

Vangl2 (also known as Strabismus 1 or Loop-tail protein 1 homolog) is a small integral membrane protein of ~60 kDa. It works in concert with prickle to modulate PCP polarity in flies, rodents and humans. Vangl2 exerts its regulatory effects by modulating effects on the actin-based cytoskeleton, particularly involved in the embryo implantation and embryogenesis during development, and also PCP orientation of different tissues and/or organs in adult animals such as stereociliary bundles in the cochlea of the inner ear in rodents and humans [36–38]. Indeed, Vangl2 (and also Vangl1) are expressed by Sertoli and germ cells in the testis [10]. Vangl2 is expressed in the seminiferous epithelium at virtually all stages of the epithelial cycle in the rat testis, co-localizing with F-actin at the apical and the basal ES, the ultrastructures that are involved in apico-basal polarity and PCP. When Vangl2 was knockdown in the testis in vivo by transfecting the testis with Vangl2-specific siRNA duplexes using the Polyplus in vivo-jetPEI transfection medium *vs.* non-targeting negative control siRNA duplexes, the status of spermatogenesis was grossly disrupted [10]. First, following knockdown of Vangl2 by RNAi, spermatids displayed signs of defects of polarity in which their heads no longer pointed toward the basement membrane but deviated by 90° to 180° of their intended orientation [10]. More important, the spatial expression of F-actin at the apical ES was grossly disrupted as F-actin appeared as bulb-like structures located predominantly at the concave side of spermatid heads in control testes [10]. However, following Vangl2 knockdown, F-actin moved away from spermatids, which was the result of changes in spatial expression of actin barbed-end capping and bundling protein Eps8 and branched actin polymerization inducing protein Arp3 [10], such that F-actin no longer prominently expressed at the apical ES to support its function. This mis-localization of F-actin thus impeded apical ES adhesion protein function since the apical ES proteins β-integrin, nectin 3 and laminin-γ3 chain all utilized F-actin for their attachment. In short, these three apical ES proteins no longer tightly associated with apical ES surrounding the spermatid heads, but mis-localized and considerably down-regulated at the site [10]. Second, the organization of F-actin across the Sertoli cell cytosol after Vangl2 knockdown was grossly disrupted since they no longer stretched across the entire Sertoli cell to support cell adhesion function, actin filaments became extensively truncated and mis-aligned [10]. Third, it was noted that following Vnagl2 knockdown in the testis in vivo, the frequency of meiosis I/II across the seminiferous epithelium in stage XIV tubules was considerably reduced, by as

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much as ~60–70%, due to the disruption of F-actin organization. Since the meiotic bundles that support chromosomal segregation during meiosis also modulated by MTs, we speculated that Vangl2 and other PCP proteins might also modulate MT dynamics. Indeed, a recent report has shown that a knockdown of Vangl2 in the testis in vivo perturbed spermatid PCP when visualized by confocal microscopy [9]. This disruption effect on MT organization following Vanlg2 known appeared to be mediated by changes in the spatial expression of MARK2 in Sertoli cells [9], a MT regulatory protein known to be involved in MT dynamics by promoting MT catastrophe [21]. Taking collectively, these findings thus support the notion unequivocally that Vangl2 that supports spermatid polarity is mediated through changes in F-actin and MT% organization.

Concluding remarks and future perspectives

While the role of actin- and/or MT-based cytoskeletons to support spermatid polarity and spermatid PCP is a rapidly developing field, there are emerging evidence, as briefly discussed herein, to support the involvement of cytoskeletons and cell polarity in the testis. However, much work is needed in the years to come. For instance, the role of Par-, Crb3- and Scribble-based apico-basal polarity protein complexes in modulating MT-organization remains largely unexplored. Also, besides Vangl2, the roles of Prickles, Dishevelled, and Frizzled proteins, in particular how Frizzled/Dishevelled complex *vs.* Vangl2/Prickle are working in concert with each other to modulate F-actin- and MT-based cytoskeletons including the downstream signaling molecules are not known. It is anticipated many of these questions will be answered in the near future. This information will provide a better picture to relate these findings to the biology of spermatogenesis in particular the functional cross-talk between polarity proteins and cytoskeletons in the testis to support germ cell differentiation and development.

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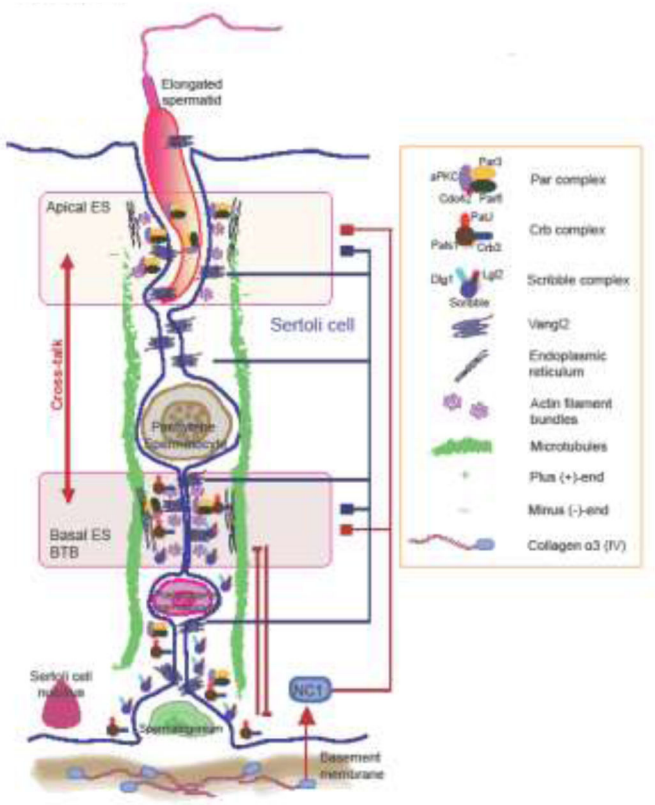

Figure 1. A schematic drawing illustrating the functional relationship between the apical and basal ES in the seminiferous epithelium of adult rat testes

As noted herein, the ES is supported by conspicuous actin filament bundles, either at the Sertoli-spermatid interface (step 1–8 spermatids in the rodent testis) or at the Sertoli cell-cell interface, known as the apical or the basal ES, respectively. The basal ES, which together with the TJ constitute the blood-testis barrier (BTB), which divides the epithelium into the apical (adluminal) and the basal compartments. The relative distribution of the Par-, the Scribble, and the Crb3-based polarity protein complexes and their corresponding partner proteins that confer the apico-basal polarity of individual spermatids in the seminiferous epithelium to support spermatogenesis are shown. Besides these polarity proteins, PCP proteins, such as Vangl2, are also present to support the alignment of polarized spermatids across the plane of the seminiferous epithelium, conferring PCP to the developing spermatids in the testis. Studies discussed in the text have shown that there are cross-talks between apical and basal ES, but more importantly that are also cross-talks between apical and basal ES through the action of Vangl2 but also NC1 domain peptide as noted in recent reports discussed in text. In short, both NC1 domain peptide generated from collagen α3 (IV) chain, a structural component of the basement membrane, and/or Vangl2 expressed by Sertoli and/or germ cells can modulate the actin- and/or MT-based cytoskeletons, thereby modulating the apical and/or basal ES function, modulating spermatid apico-basal and/or PCP polarity.

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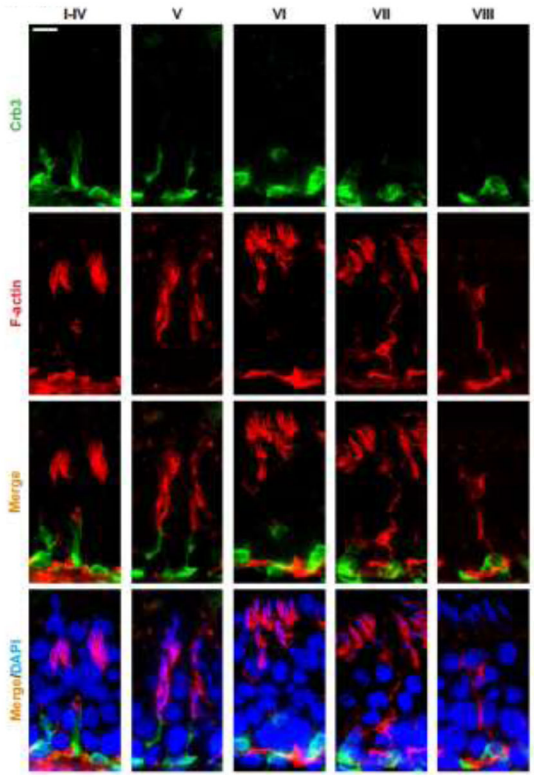

Figure 2. A study that illustrates co-localization of polarity protein Crb3 and F-actin in the seminiferous epithelium of adult rat testes

Crb3 (green fluorescence) and F-actin (red fluorescence) were visualized in the seminiferous epithelium of adult rat testes using corresponding specific antibody and/or reagents as earlier described [6, 39]. It was noted that Crb3 only partially co-localized with F-actin in the epithelium during the epithelial cycle of spermatogenesis. Cell nuclei were stained by DAPI (4′,6-diamidino-2-phenylindole). Scale bar, 40 μm, which applies to all other micrographs.

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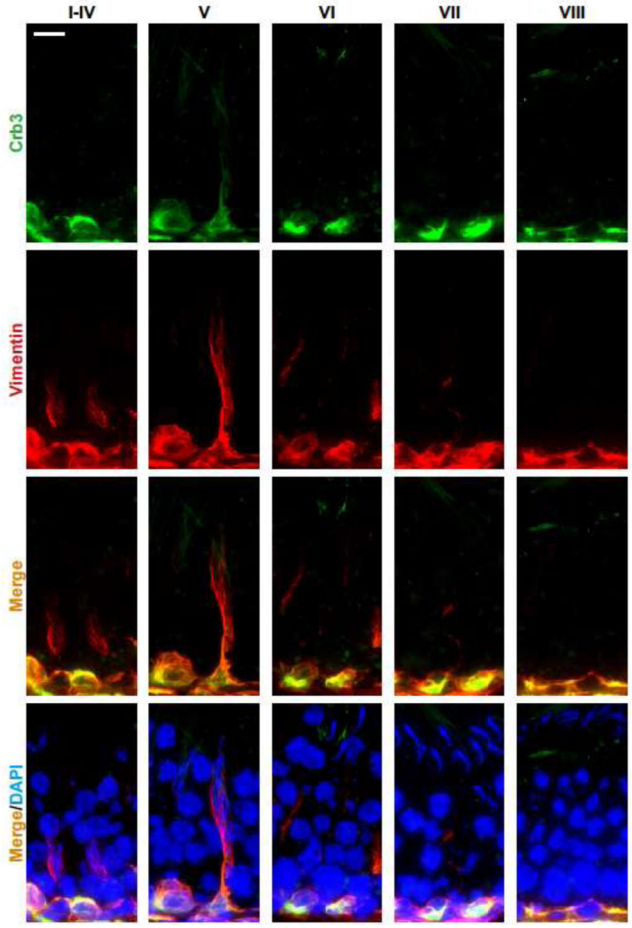

Figure 3. A study that illustrates co-localization of polarity protein Crb3 and vimentin in the seminiferous epithelium of adult rat testes

Crb3 (green fluorescence) and vimentin (red fluorescence) were visualized in the seminiferous epithelium of adult rat testes using corresponding specific antibody and/or reagents as described [6, 39]. It was noted that Crb3 co-localized with F-actin, almost superimposable, in the epithelium during the epithelial cycle of spermatogenesis. Cell nuclei were stained by DAPI (4′,6-diamidino-2-phenylindole). Scale bar, 40 μm, which applies to all other micrographs.

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Table 1

Functions of different polarity proteins and PCP proteins in mammalian cells and tissues.

	Protein	Phenotype in rodents following deletion (knockout, KO) or knockdown (KD) in corresponding model	References	Mutation(s), deletion or changes in expression that lead to corresponding diseases in humans	References
Par3-Complex	Par3			Up-regulation in ovarian and prostate cancer, down-regulation in pancreatic cancer.	[40–42]
	Par6			Up-regulation in breast cancer and non-small-cell lung cancer, mutation inhibits heart development.	[43–45]
	Cdc42	Cdc42-deficiency causes forebrain malformation, failing to develop into two hemispheres, leading to holoprosencephaly.	[46]	Up-regulation in polycystic kidney disease, mutation leads to thrombocytopenia.	[47, 48]
	PKC	PKCι knockout leads to embryonic fatality, conditional deletion of aPKCλ in differentiated neurons causes polarity complex disruption.	[49, 50]	Mutation leads to Alzheimer's disease; Down-regulation in B-cell chronic lymphocytic leukemia.	[51, 52]
Scribble-Complex	Scribble	Deletion leads to embryonic fatality, mutation leads to lung and prostate cancer.	[53–55]	Mutation leads to lung cancer, down-regulation in prostate cancer.	[54, 55]
	Dlg1	Deletion leads to embryo0nic fatality, requires for development of respiratory, cardiovascular and urogenital systems.	[56–58]	Mutation leads to Crohn's disease, and schizophrenia.	[59, 60]
	Lgl2			Mutation leads to Barrett gastric foveolar dysplasia, a congenital gastroesophageal reflux disease.	[61]
Crumbs-3-Complex	CRB3	Deletion leads to embryonic fatality, requires for the development of kidney and lung.	[62]		
	PALS1	PALS1 shRNA knockdown in developing brain leads to the presence of excessive neurons, and followed by massive apoptosis, causing abrogation of the entire cortical structure; conditional PALS1 knockdown in mouse E14 embryonic stem cells causes defects in retina.	[63, 64]		
	Vangl2	Deletion in mice perturbs brain development, leading to embryonic fatality in some, but not all, mice.	[65]	Mutation leads to congenital heart defect and neural tube defects.	[66, 67]
PCP Complex	Prickle1	Deletion leads to embryonic fatality due to failure of distal visceral endoderm migration.	[68]	Mutation leads to neural tube defects and type 2 diabetes.	[69, 70]
	Dvl3	Deletion leads to embryonic fatality due to defects in heart formation.	[71]	Mutation leads to: (i) Robinow syndrome manifested by short-limbed dwarfism and abnormalities in the head, face and external genitalia; (ii) prostate cancer; (iii) leukemia; (iv) microcephaly; (v) depression; (vi) Hirschsprung's disease; or (vii) lung cancer.	[72–78]
	Fzd3	Embryonic fatality, essential for the brain development.	[79, 80]	Mutation leads to schizophrenia, and Hirschsprung disease (due to the absence of nerve cells in colon, leading to chronic constipoation); up-regulation in polycystic kidney disease.	[47, 81, 82]